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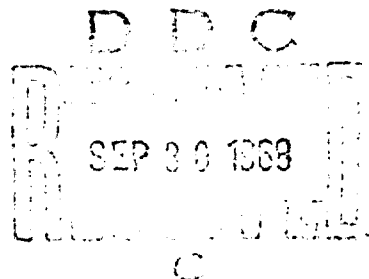| AUTHORITY |
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METHOD OF ACTION OF BACTERIAL ENDOTOXINS

I.   INTRODUCTION TO A GENERAL STUDY

A. DELAUNAY and J. LEBRUN

I.   One of the essential components of gram-negative bacteria is
given the name of *bacterial endotoxins*. Various chemical methods have
been developed for isolating them from these bacteria. The first in
date, which is also the most practical one -- although not the least
rough one -- is A. Bolvin and L. Mesrobeanu's method (precipitation of
the bacteria with trichloroacetic acid). Regardless of the method used,
the endotoxin is finally obtained in the form of a powder easily soluble
in distilled water or in physiological water. Experiments performed in
the laboratory almost always use aqueous solutions of endotoxins.

Today, the chemical structure and the various properties of bac-
terial endotoxins are quite well known.[1] From the chemical point of
view, they are made up of special complexes that always contain a spe-
cific polysaccharide (this polysaccharide is what ensures the specifi-
city of an endotoxin), to which sometimes lipid substances, sometimes
polypeptides, are linked, most often both lipids and polypeptides. At
the present time, it is not yet known precisely whether these complexes
represent a particular fragment of the bacterial cells or whether they
only form non-differentiated and more or less voluminous parts of the
microbes concerned. However, the second hypothesis appears to be the
more likely.[2]

Endotoxins have, in common with all microbian toxins, two great
properties. They are antigenic and they are toxic. We shall say no-
thing here about their antigenic power, which is unquestionable. We shall
concentrate all our attention on their toxic power.

II. Endotoxins do not seem to be very toxic to cold-blooded animals.[3] We were able to inject several milligrams, even several multiples of ten milligrams, of typhus endotoxin in frogs, grass-snakes and fish without killing them. Therefore, these animals are resistant to every microbian poison, regardless of its nature: proteinic (exotoxins) or gluco-lipo-polypeptidic (endotoxins).

On the other hand, endotoxins have proved to be toxic to every mammal tested so far: mice, rats, guinea-pigs, rabbits, sheep, goats, horses, asses, dogs, pigs, etc., and to birds. However, the lethal dose appears to vary considerably from one case to another. Among birds, for example, the pigeon proves to be much more resistant than the chicken.[4] Important differences in toxicity to mammals are also observed.

Thus, *mice* and *guinea-pigs* are very sensitive to endotoxins. One tenth of a milligram of poison is sufficient to kill a 20-gram mouse in a few hours. The lethal dose for a 300-gram guinea-pig is of the nature of one milligram. It is generally believed that *rabbits* are irregularly sensitive. At times, very weak doses kill them, and, at times, high doses only induce passing disturbances in them. Weight for weight, *rats* prove to be more resistant than mice. In order to induce death in a few hours in normal rats weighing 80 grams, we had to inject 6 to 8 milligrams of typhus endotoxin subcutaneously.[5] Finally, *man* seems to be most particularly sensitive, since one thousandth of a milligram of the same toxin, introduced in his skin, causes an extremely marked local reaction, and sometimes even a violent general reaction.[6]

The figures that we have just given are interesting, for they show that, weight for weight, endotoxins are much less toxic than exotoxins. It is known, in fact, that one ten-thousandth of a milligram of diphtheria protein-toxin is sufficient to kill a guinea-pig.

Of course, the dose of endotoxin injected proves to be all the more rapidly lethal the higher it is, and it is a commonly observed fact that intravenous and intraperitoneal injections are more dangerous than subcutaneous injections. In turn, an intracutaneous injection is probably more dangerous than a subcutaneous one.

However, right here several *fundamental* remarks, to which we shall frequently have occasion to refer later, must be made here.

I. Regardless of the germ furnishing it -- *Salmonella, colibacilli*, etc. -- an endotoxin always causes the same picture of intoxication in a sensitive organism. For a given amount of poison, there is no difference between the clinical picture that follows the injection, for example, of a cholera endotoxin in an animal and the clinical picture occurring in an animal treated with a typhus endotoxin. This similarity of action of the two products is striking. Let us recall that the states of intoxication caused by all exotoxins are, on the other hand,

characteristic of them.

2. The toxic power of any endotoxin whatsoever operates with extremely great rapidity. No incubation time is observed here. Less than an hour after the subcutaneous injection of a simply sublethal dose of the poison, clear signs of intoxication are already found. The state of intoxication rapidly becomes worse. In a guinea-pig treated with a strong dose it is deepest at the end of five to eight hours. At this time, two eventualities may occur: either the animal dies, or it rapidly regains a normal state. It may be said that, as a general rule, an animal that has been able to hold out for twenty-four hours against the harmful action of the endotoxin is saved. This remark seems to us to be of primary importance. We shall soon show why.

3. Weak and repeated does of an endotoxin often spare the animal's life, but cause a very appreciable state of emaciation. This fact, which was well observed by Professor Lisbonne shortly before his death (personal communication), has not yet been explained.

Under certain conditions, an endotoxin is capable of losing part or all of its toxic power.

This loss may be owing to a modification of the endotoxin itself. Thus, an endotoxin that has been kept under refrigeration for months always turns out to be less harmful than at the time of its preparation. It is probably also possible to attenuate or eliminate the toxicity of an endotoxin by acetylation.[7]

More often, a decrease in the toxicity of the product merely expresses an increase in the organism's resistance. The subject's age appears to have no effect whatsoever on the value of this resistance.[8] On the contrary, it probably depends, to a certain extent, on the animal's state of nutrition. Thus, the toxicity is greater, when the tissues are rich in ascorbic acid.[9] Finally, it is possible to strengthen very definitely immunity against endotoxins by means of a specific[10], and even non-specific[11], immunization against endotoxins.

In addition, various studies seemed to show that sulfonamides[12] and penicillin[13] are probably capable of neutralizing the harmful action of these poisons, at least to a certain point.

In any case, this same action may be conveniently attenuated by introducing the products into the organism in a mixture with an adjuvant substance.[14] We have had many opportunities to verify this fact personally.

Several authors have wondered whether the toxicity of the gluco-lipo-polypeptidic complexes was linked to the entire complex or only to a part of this complex. So far, their studies have only led to contradictory results.[15]

III. Let us now examine the lesions discovered in animals that died after an injection of endotoxin. They have been described very often.

After abdominal laparotomy, we are struck by the appearance of the small intestine, which is distended by a yellowish, often hemorrhagic, liquid and which contains in suspension numerous epithelial cells that are more or less altered, isolated or assembled in layers (desquamation). The Peyer's patches are very heavily congested (in the rabbit they stand out like small raspberries on the intestinal mucous membrane)[16], and the mesenteric ganglia are swollen, often even hemorrhagic.[17] The spleen has increased in volume. Local foci of congestion and edema, and hemorrhages are also found on the lungs at the opening of the thorax.

All these lesions show up still more clearly on microscopic examination of the tissues. The capillaries of the lungs are dilated and full of neutrophils and lymphocytes.[18] Edema, hemorrhages and necrosis foci (often lacking any infiltration by polymorphonuclears)[19] are found in the liver. Congestion and edema are also very evident in the spleen, whose sinuses are dilated, in all the ganglia, in the cardiac muscle, in the kidneys, in the brain, in the spinal cord, etc. Vascular thromboses are extremely frequent at every point.[20] Congestive and hemorrhagic foci are particularly large in the suprarenal capsules.[21] As an interesting fact, a rapid disappearance of the chromaffin substance has been observed in poisoned animals.[22] Finally, a congestion and hyperplasia of the bone marrow have been noted.

The hemorrhagiparous tendency of bacterial endotoxins was well demonstrated by the studies of Zahl and his colleagues.[23] It is known that, when certain filtrates of microbian cultures are injected in animals that have tumors, they very often cause hemorrhages in these tumors and, at the same time, sometimes bring about their complete regression.

Zahl was able to identify the bacterial substances, active in this case, with endotoxins. Actually: a. the most powerful preparations are, as a general rule, the ones that come from gram-negative bacteria; b. they also are the ones that contain endotoxins; c. finally, endotoxins by themselves are perfectly capable of causing hemorrhages in tumors.

During their studies, the authors found some exception, however. For example, the toxic extracts of some gram-negative bacteria -- among others the plague bacillus -- are not hemorrhagiparous. On the contrary, certain extracts of gram-positive bacteria -- some strains of Listerelia -- may sometimes produce intratumoral hemorrhages, although they contain no endotoxin.

Several observations have demonstrated the frequency of abortions in subjects afflicted with typhoid fever or cholera.[24] According to all likelihood, these accidents result from retroplacental hemorrhages caused

by the endotoxins of the infecting bacteria.[25]

We shall conclude this enumeration of the lesions caused by endo-
toxins by pointing out that these poisons may determine the appearance
of tumors in plants.[26]

IV. An important question is now put to us. How are these le-
sions caused? Since it is primarily a question of hemorrhagic lesions
(edema, congestion, more or less large hemorrhages, thromboses and subse-
quently necroses), must endotoxins be considered as vascular poisons?
Numerous authors have thought so.[27]

However, it appears to us difficult to admit that here we are
faced with a *direct* attack on the vessels by the endotoxins. Actually,
the very numerous experimental data collected during these last few years
are all in agreement to show that endotoxins do not have a pronounced
irritant power for cells, regardless of what cells may be involved.

a. At any rate, they are not leukocidins. Polymorphonuclears
that have been kept for hours in a serum medium containing a large
amount of endotoxin remain capable of moving towards the chemical sub-
stances that normally attract them,[28] of phagocytizing[29] and of breath-
ing[30]. On the contrary, the respiratory metabolism of these cells appears
to be increased slightly by contact with an endotoxin. The other phago-
cytes -- histiocytes, macrophages and cells of the reticuloenothelial
system -- do not appear to be harmed by the same bacterial poison either.
They continue properly to phagocyticize foreign microbes and colloids,
even within organisms deeply poisoned by endotoxins.[31]

Moreover, endotoxins have no hemolytic power.

b. In addition, the action of these same products on the respira-
tory metabolism of different cells has been studied. While E. Boyland
and M. E. Boyland were observing some fragments of experimental rat tu-
mors, they noted a slight reduction in respiration, when the fragments
were put in the presence, *in vitro*, of filtrates of Eberth's bacillus.[32]
On the other hand, according to M. E. Delafield and H. A. Smith, the en-
dotoxin of Aertryck's bacillus introduces no disturbance in the normal
consumption of oxygen by cerebral or muscular cells.[33]

c. The experiments made on tissue cultures seem still more in-
teresting to us. Barg observed, for example, that an extremely large
amount of Shiga's bacillus toxin was needed to inhibit the growth of
tissues *in vitro* and that, even under those conditions, the lesions
caused had nothing specific.[34]

In the same order of ideas, the results obtained by C. J. Shapi-
ro are worth pointing out.[35] Since this author's attention had been
struck by the hemorrhagic and necrotizing action of a polysaccharide

extract of *S. enteritidis* on rat tumors, he attempted to find the same action of the extract on young cancerous cells cultivated *in vitro*. But he failed. The bacterial extract turned out to be without action *in vitro*, regardless of whether it was added to the culture alone or in a mixture with the plasma of cancerous rats.

Our personal results agree perfectly with the findings of Barg and Shapiro.[36] While, with a definitely necrotizing toxin *in vivo*, like the staphylococcic exotoxin or the exotoxin of *B. perfringens*, it proves to be very easy to kill cells *in vitro* directly -- and even by resorting to relatively weak doses of poison --, we had to use very strong doses of typhus endotoxin and operate under very violent conditions to disturb the development of our cultures of fibroblasts-macrophages.

According to all these results -- and also taking into account the rapidity with which these lesions are produced in the organism -- it appears to be quite impossible to make the lesions observed in the intoxicated animals depend on a *direct* attack on the tissues, in the manner of a caustic, by endotoxins.[37] Therefore, it is necessary to look in another direction for the pathogenesis of these lesions.

V. Then a plausible explanation comes to mind. Do not the lesions in question result from certain humoral and nervous disturbances that originate in the poisoned subjects? *A priori*, the fact seems likely. Let us see what the nature of these disturbances might be.

1. *Is it a question of a humoral reaction?* A. Penner and A. I. Bernheim[38] thought so. According to them, the endotoxin, injected into the organism, probably is limited to bringing into play a hypersecretion of adrenalin. When this hormone is released abruptly and in high doses, it probably causes a generalized arteriospasm which suffices to account for all the vascular manifestations then observed: edema, congestion and hemorrhages. This point of view has received so far -- at least to our knowledge -- neither invalidation nor confirmation. As far as we are concerned, we do not think that the state of intoxication caused by an endotoxin can be entirely reduced to a simple state of adrenalitic shock. Nevertheless, the conception of the American authors is interesting, for it contributes to attracting attention to the possible disturbances of the neurovegetative system in animals treated with an endotoxin.

2. It really can hardly be doubted any longer that this *neurovegetative system* is capable of reacting in contact with the endotoxin (by introducing *ipso facto* serious disorders in the organism.

a. Thus, in 1940, P. Gastinel and J. Reilly[89] were able to cause considerable congestive lesions to the intestine, a hypertrophy of Peyer's patches, an infarction of the meseteric ganglia, merely by depositing a small amount of typhus endotoxin on the splanchnic nerve or by injecting

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the poison into the mesenteric ganglion.

b. On his part, R. Laplane[40] succeeded, in 1945, in causing typical arteritis lesions experimentally with the typhus endotoxin. Now, for Laplane, everything indicates that the endotoxin caused those lesions, not directly, but by irritation of the adventitial sympathetic system.

c. We shall also mention the studies made by G. Tardieu[41]. When this author injected dogs ventricularly with weak doses of typhus endotoxin, he reproduced in the animals the principal symptoms of typhoid fever, that is to say, stupor, disturbances of the tonus, hyperthermia, renal signs, emaciation, diarrhea, etc.

d. Finally, during these last few years, several observations have come along to emphasize the frequency of an attack on the nervous system during typhoid fever. In particular, numerous cases of typhic encephalitis have been found recently in the south of France.[42] Since some of these cases have had a fatal outcome, it has been possible to make an accurate histological examination of the patients' brains.[43] This examination brought to light, particularly in the hypothalamus area, extremely definite circulatory reactions (edema, congestion, hemorrhages), accompanied by a degenerescence of the glial and neuronal cells and by neuroglial reactions.

e. The possibility of an interference with the neurovegetative system in the genesis of the lesions that we are studying here has also been taken into account abroad. In this respect, we shall mention the recent study by P. A. Zahl and S. H. Hutner.[44] According to those two Americans, the modalities of this interference might differ, moreover, from one animal species to another. In the mouse and the guinea-pig, the endotoxin probably stimulates primarily the parasympathetic system. Let us add here that, according to Professor Topley, an injection of insulin often aggravates the state of intoxication produced in the mouse by an endotoxin.[45] On the other hand, in the rat, the cat, the rabbit and man, it is probably basically a question of a sympatheticotonia. Zahl and Hutner have adduced a certain number of arguments in support of their thesis. We shall have nothing to say about them here; we shall await an opportunity to discuss them in detail subsequently.

VI. Very briefly set forth[46], these are the main facts that have already been assembled on the method of action of endotoxins. We can summarize them as follows:

1. Bacterial endotoxins behave like toxic substances for mammals.

2. In animals whose death they have caused, we are especially struck by the lesions that express an attack on the vessels, edema, congestion, hemorrhages.

3. In all likelihood, these lesions are not secondary to a direct change in the walls of the vessels by the endotoxin.

4. They are, rather, the work of an excessive reaction by the organism in contact with the poison.

But what is the exact nature of this reaction?

These questions have not yet been well elucidated. We have been devoted to solving them since 1941. Today we are beginning to publish the results that we have obtained.

FOOTNOTES

[1] In this connection, see: A. BOIVIN, I. MESROBEANU and L. MESROBEANU, *C. R. Soc. Biol.*, 1933, 114, 307; 1934, 115, 306. -- A. BOIVIN and L. MESROBEANU, *Rev. Immunol.*, 1935, 1, 553; 1936, 2, 113; *La Presse medicale*, 1936, No 99, 1995; Sixth Biological Chemistry Congress, 1937, Lyons (volume of the Congress, 401); *Annales de l'Institut Pasteur*, 1938, 61, 426. -- A. BOIVIN, *Paris Med.*, 1939, 29, 133. -- *Exposés annuels de Biochimie medicale* (Prof. POLONOVSKI), 3rd series, 1942, 113. -- *Rapport au Congres de Microbiol.*, Basle, 1946 (in press), etc.

[2] In this connection, see: A. BOIVIN and A. DELAUNAY, *Bull. Acad. Med. 1944*, 128, 357 and *C. R. Soc. Biol.*, 1944, 138, 468. -- A. BOIVIN, A. DELAUNAY and Y. LEHOULT, *C. R. Soc. Biol.*, 1944, 138, 398 and 724, etc.

[3] A. DELAUNAY, P. BOQUET and J. PAGES, *C. R. Soc. Biol.*, 1945, 139, 993.

[4] E. GRASSET, A. ZOUTENDYKE and A. SCHAAFSMA, *Brit. J. exp. Path.*, 1935, 16, 454.

[5] A. DELAUNAY, J. PAGES and M. MARTINET, *C. R. Acad. Sci.*, 1946, 223, 218.

[6] R. KOURILSKY, S. KOURILSKY and A. BOIVIN, *C. R. Soc. Biol.*, 1939, 131, 190.

[7] H. P. TREFFERS, *Science*, 1946, 103, 387.

[8] P. A. ZAHL, S. H. HUTNER and F. S. COOPER, *Proceed. Soc. Exp. Biol. and Med.*, 1943, 54, 137.

[9] H. A. SCHNEIDER and L. T. WEBSTER, *J. Exp. Med.*, 1945, 81, 359. -- H. B. ANDERVONT and H. B. SHIMKIN, *American J. Cancer*, 1939, 36, 451.

[10] In this connection, see: A. BOIVIN and L. MESROBEANU, *Rev. Immunol.*, 1938, 4, 40 and 197. -- A. BOIVIN, R. KOURILSKY and S. KOURILSKY, *C. R. Soc. Biol.*, 1939, 131, 190, etc.

[11] P. A. ZAHL, S. H. HUTNER and F. S. COOPER, *Proceed. Soc. Exp. Biol. and Med.*, 1943, 54, 187. -- P. A. ZAHL and S. H. HUTNER, *Amer. J. Hyg.*, 1944, 39, 189; *Proceed. Soc. Exp. Biol. and Med.*, 1944, 55, 134.

[12] Among the most recent studies, we shall mention a study by P. A. ZAHL, S. H. HUTNER and F. S. COOPER, *Proceed. Soc. Exp. Biol. and Med.*, 1944, 55, 4.

[13]A. K. BOOR and C. P. MILLER, *Science*, 1945, 102, 437. -- C. P. MILLER and A. K. BOOR, *Proceed. Soc. Exp. Biol. and Med.*, 1946, 61, 18.

[14]In this connection, see, for example: S. P. HALBERT, J. SMOLENS and ST. MUDD, *Amer. J. of Med. Sciences*, 1945, 209, 411.

[15]A critical analysis of these studies will be found in the report submitted by A. BOIVIN to the Microbiology Congress at Basle (1946) and entitled: "Recent Studies on the Chemical Composition and on the Biological Properties of Bacterial Antigens."

[16]A. BOIVIN, *Exposes annuels de Biochimie medicale* (Dr. Polonovski), 3rd series, 1942, 113.

[17] R. KOURILSKY, S. KOURILSKY and J. BABLET, *C. R. Soc. Biol.*, 1941, 135, 18.

[18]R. C. ROBERTSON and H. Yu, *J. of Hygiene*, 1938, 38, 299.

[19]E. W. DENNIS and H. SENEKJIAN, *Amer. J. of Hygiene*, 1939, 30, 21.

[20]H. R. MORGAN, *Amer. J. of Path.*, 1943, 19, 135.

[21]A. R. MARTIN, *Brit. J. Exper. Path.*, 1934, 15, 137.

[22]E. W. DENNIS, *Proceed. Soc. Exp. Biol. and Med.*, 1939, 42, 553. -- Concerning lesions of the suprarenal glands, also consult L. OLITZKI, S. AVINERY and P. K. KOCH, *J. Immunol.*, 1942, 45, 337.

[23]P. A. ZAHL, S. H. HUTNER, S. SPITZ, S. KANEMATZU and F. S. COOPER, *Amer. J. of Hygiene*, 1942, 36, 224. -- P. A. ZAHL and S. H. HUTNER, *Proceed. Soc. Exp. Biol. and Med.*, 1942, 51, 285; 1943, 52, 116 and 364. -- P. A. ZAHL, S. H. HUTNER and F. S. COOPER, *Proceed. Soc. Exp. Biol. and Med.*, 1943, 54, 48. -- P. A. ZAHL, M. P. STARR and S. H. HUTNER, *Amer. J. of Hygiene*, 1945, 41, 41, etc.

[24]G. SANARELLI, *Ann. Inst. Pasteur*, 1924, 38, 11. -- A. F. JENNINGS and D. R. MATHIESON, *Practice of Medicine*, 1940 (W. F. Prior Co., Hagerstown, Md.), etc.

[25]P. A. ZAHL and Cl. BJERKNES, *Proceed. Soc. Exp. Biol. and Med.*, 1943, 54, 329 and 1944, 56, 153, etc.

[26]A. BOIVIN, M. MARBE, L. MESROBEANU and P. JUSTER, *C. R. Acad. Soi.*, 1935, 201, 984. -- A. BOIVIN, L. MESROBEANU, M. MARBE, P. JUSTER and T. SAVULESCO, *Arch. Roum. Path. Exp. Microb.*, 1937, 10, 67, etc.

[27]In this connection, see particularly: P. A. ZAHL, S. H. HUTNER and F. S. COOPER, *Proceed. Soc. Exp. Biol. and Med.*, 1943, 54, 137.

[28]A. DELAUNAY, *C. R. Soc. Biol.*, 1944, 138, 27. -- E. Lasfargues and A. DELAUNAY, *C. R. Soc. Biol.*, 1946, 140, 455.

[29]A. DELAUNAY, *C. R. Soc. Biol.*, 1942, 136, 729. -- *Rev. Immunol.*, 1942, 7, 208.

[30]J. PAGES and A. DELAUNAY, *C. R. Acad. Soi.*, 1946, 222, 155.

[31]A. DELAUNAY, R. SARCIRON and J. PAGES, *C. R. Soc. Biol.*, 1944, 138, 345.

[32]E. BOYLAND and M. E. BOYLAND, *Biochem. J.*, 1937, 31, 454.

[33]M. E. DELAFIELD and H. A. SMITH, *Brit. J. of Exp. Path.*, 1936, 17, 379 and 1939, 20, 216.

[34]Quoted by A. PENNER and A. I. BERNHEIM, *J. of Exp. Med.*, 1942, 76, 271.

[35]C. J. SHAPIRO, *Amer. J. of Hygiene*, 1940, 31, 114.

[36]A. DELAUNAY and E. LASFARGUES, *C. R. Soc. Biol.*, 1945, 139, 363. -- E. LASFARGUES and A. DELAUNAY, *Ann. Inst. Pasteur*, 1946, 72, 38.

[37]The possibility is not excluded, however, that endotoxins may exercise a relative toxicity with regard to certain cells by combining, for example, with some cellular diastase and by disturbing the metabolism of the components struck by that very fact.

[38]A. PENNER and A. L. BERNHEIM, *J. of Exp. Med.*, 1942, 76, 271.

[39]P. GASTINEL and J. REILLY, *C. R. Soc. Biol.*, 1940, 134, 456.

[40]R. LAPLANE, *Bull. Soc. Med. Hop.*, Paris, Nos. 11 and 12, 134.

[41]G. TARDIEU. *La Presse Medicale*, 1942, Nos. 7-8, 75.

[42]J. A. CHAVANY, E. BODET and J. RAIMBAULT, *La Presse Medicale*, 1943, No. 12, 147. -- H. ROGER and H. GASTAUT, *La Presse Medicale*, 1945, No 52, 709. -- R. DAMADE and A. LAVIGNOLLE, *Gaz. des Hop.*, 1946, No. 3, 49. -- M. SCHACHTER, *Bull. Med.*, 1946, No. 28, 353. -- CH. SARROUY, P. COMBE, R. ARNAUD-BATTANDIER and TABONE, *Algerie Med.*, 1946, No. 5, 449. -- E. BENHAMOU, F. DESTAING and LEONARDON, *Algerie Med.*, 1946, No. 5, 452, etc.

[43]L. CORNIL, H. ROGER, Y. POURSINES and H. GASTAUT, *Sem. Hop.*, Paris, 1946, No. 16, 681.

[44]P. A. ZAHL and S. H. HUTNER, *Proceed. Soc. Biol. and Med.*, 1944, 56, 156.

[45]Quoted by A. R. MARTIN, *Brit. J. of Exp. Path.*, 1934, 15, 137.

[46]We have been able to give here only a rather incomplete bibliography on the subject. It will be developed extensively in the Sciences thesis by one of us (J. LEBRUN).